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Sir:

Transmitted herewith for filing is the patent application of

Inventor/Owner: **SCHNEIDER, James R.**
For: **A PRESERVED IMPLANTABLE VESSEL DERIVED FROM A HUMAN UMBILICAL CORD**

Enclosed are:
[X] **1** sheet of **informal** drawings.
[ ] An assignment of the invention to _____.
[ ] A certified copy of a _____ application.
[ ] ____ Specimens (2 minimum)
[X] A Verified Statement to establish small entity status under CFR 1.9 and 37 CFR 1.27.
[X] Declaration/Statement and Power of Attorney.
[X] Specification and Claims.
[X] **9** copies of prior art references listed on the PTO 1449 Form.

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Date: August 10, 1998

THEODORE J. BIELEN, JR.
Reg. No. 27,420

PATENT

JAMES R. SCHNEIDER

SPECIFICATION AND CLAIMS
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FOR
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LETTERS PATENT
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FOR
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A PRESERVED IMPLANTABLE VESSEL DERIVED FROM A HUMAN UMBILICAL CORD

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A PRESERVED IMPLANTABLE VESSEL DERIVED FROM A HUMAN UMBILICAL CORD

ABSTRACT OF THE DISCLOSURE

A preserved vessel isolated from a human umbilical cord and lyophilized for use as an allograft which improves blood supply to human tissue without antigenicity.

BACKGROUND OF THE INVENTION

The present invention relates to a novel vascular allograft.

Since about the year 1900, much research effort has been undertaken to provide substitute vascular conduits in surgical situations. In essence, a substitute or artificial vein for the natural vein in a mammalian species provides oxygen to blood tissues and removes venous blood from the tissues in the same manner. Many situations have been identified where substitute vessels are needed:

1. Replacement vessels are needed for supplying ischemic tissue, which cannot obtain enough oxygen and nutrients to stay alive. Such a vessel may be required in cases of injury to a blood vessel which cannot be primarily repaired, or when a vessel is excised as a result of tumor mass or other obstructions.

2. A bypass vessel may be employed to circumvent an obstruction and may be emplaced by an end-to-side connection. Thus, blood is permitted to flow around the obstruction through this expedient.

3. Collateral vessels may be employed to provide circulation to ischemic tissue without disrupting inefficient vessels.

4. A shunt may be devised, such as one needed for renal dialysis. For example, a vessel may be placed in the forearm of a human in a subcutaneous position to permit venepuncture allowing dialysis to proceed.

5. A vessel extender may be provided in cases of free tissue transfer. In other words, such extenders may be employed where the existing pedicle of the composite graft is not sufficiently long.

In general, the mammalian circulation system operates when the heart pumps blood carrying oxygen and nutrients through arteries to all of the tissues of the body. Subsequently, oxygen-deprived blood and metabolic byproducts are returned by the veins to replenish oxygen for passing such metabolic byproducts to waste. The veins have lower blood pressure than the arteries. Thus, the blood returns to the heart by back pressure from the arterial system, muscular action in the limbs, and gravity in the case where portions of the body lie above the heart. Low venus blood from the lower limbs is facilitated by one-way valves in the walls of the veins. For example, the saphenous veins permit flow toward the heart but prevent backflow therefrom. Thus, veins in this aspect constitute a one-way flow system.

In the past, it has been found that autogenous grafting has achieved success without evidence of graft rejection. In allografts, however, tissue removed from one person and implanted in another person typically has resulted in graft rejection, requiring the use of immunosuppressive drugs. It should be noted that the use of heterografts (xenografts) has been uniformly unsuccessful due to rapid rate of graft rejection. Within the last 50 years, tissue banks supplying frozen tissue have been

established. Such banks, however, have not completely solved the problem of antigenic rejection with respect to allograft tissue.

Modified vascular conduits have been provided utilizing chemical preservatives such as glutaraldehyde in conjunction with a mesh frame work. Reference is made to United States Patents 3,988,782, 3,974,526, and 4,239,492 in which a process for producing tubular prostheses is described. Unfortunately, such preservation system is relatively short-lived.

Artificial grafts, such as ones constructed of polytetrafluoroethylene (PTFE) have proved successful when used with larger diameter vessels, but unsuccessful in smaller diameter vessels. Further, anastomosis between the PTFE graft and different sized vessels is difficult. In addition, the PTFE grafts are rigid and don't propagate the pulse within the body. Moreover, PTFE grafts tend to kink if bent beyond a certain degree.

Autologous grafts are commonly used in heart bypass surgery. That is to say, saphenous veins from human legs have been harvested and transplanted into the heart as a bypass vessel. In addition, such autologous grafts have also been used as a dialysis shunt, for free tissue transfers, and as an added vascular pedicle. Although successful in many aspects, saphenous veins are usually thin walled and hard to handle. During harvesting procedures, saphenous vessels possess tributaries which must be tied off causing flow turbulence in the patients circulation system. Also, saphenous veins have varying widths and may include one-way valves which must be eliminated. In addition, extra operating time is

required for harvesting vessels from a patient's leg prior to implantation. After harvesting, lower limb complications may occur in the patient such as delayed healing or painful scars in the leg. Moreover, arteries are not usually available for use as an autograft. Arteries harvested from a cadaver for use as an allograft have exhibited graft rejection problems. However, arteries if usable, are easier to handle because they are thicker walled, contain no one-way valves, and possess less tributaries to be tied off or isolated. It has been found historically that arteries, specifically the media layer, will spasm if used as a graft leading to occlusion of the vessel.

Lyophilization of human and other mammalian tissue has been employed to preserve bone, fascia, tendons, cartilage, ligaments, and the like. United States Patents 5,656,498 and 5,690,963 described freeze-drying of blood cells and the like for reuse. Several publications entitled Rat Epigastric Pedicle Model: A Clinically Relevant Evaluation Of 1-mm PTFE Grafts, Barttelbort et al; Microsurgical Application Of Freeze-Dried Venous Allografts, Pratt et al; Microsurgical Application Of Freeze-Dried Arterial Allografts, Pratt et al; and Experimental Freeze-Dried Microarterial Allografts In Rabbits, Pratt et al indicate that lyophilization may reduce or eliminate graft rejection in femoral arteries in rats and rabbits.

United States Patent 4,239,492 described a method of preparing vascular grafts from umbilical cords using chemical preservatives.

The provision of a preserved vessel isolated from the human umbilical cord for use as an allograft would be a notable advance in the medical field.

SUMMARY OF THE INVENTION

In accordance with the present invention a novel and useful preserved vessel from a human umbilical cord is herein provided for the purpose of implantation as an allograft.

Human umbilical cords having two arteries and one vein are used as the source for the vessels of the present invention. The vessels are isolated from the umbilical cord by blunt and sharp dissection techniques. With continuing irrigation during this process, the vessels are separated from each other and the sheath of the umbilical cord. Stents, such as plastic, i.e. nylon, stents, are placed within the lumen of the vessels to maintain the vessel diameter during the freeze-drying and rehydration phases, described hereinafter.

Each vessel and stent is placed in a moist cotton carrier and positioned within a freeze-drying canister. The canister may be refrigerated until the freeze-drying process takes place. A vacuum is applied to the canister when placed in a standard freeze-dryer for a requisite period of time and at an acceptable temperature, known in the art. It is believed that the water within the vessels being lyophilized passed from a frozen crystalline state into the vapor state by the process of sublimation. This process avoids disruption of the integrity of the cellular structure of the vessels which normally occurs during passing of liquid water into a solid state. Following freeze-drying each vessel specimen may be stored in the canister at room

temperature in its lyophilized condition and with the vacuum seal being maintained.

Rehydration of the vessels takes place by simply removing the vessels with the enclosed stents and the cotton carrier from the freeze-drying canister, and soaking the same in heparinized saline for 20 minutes. After discarding of the cotton carrier, the plastic stent is maintained within the vessel until the vessel is trim and implanted by standard vascular anastomosis techniques.

While in the foregoing it may be apparent that a novel and useful preserved vessel useful for implantation is herein provided.

It is therefore an object of the present invention to provide a preserved vessel for implantation which derives from a human umbilical cord.

Another object of the present invention is to provide a preserved vessel derived from a human umbilical cord which may be implanted as an allograft without rejection by a human entity.

Another object of the present invention is to provide a preserved vessel derived from a human umbilical cord for implantation which possesses a relatively long shelf life.

A further object of the present invention is to provide a preserved vessel derived from a human umbilical cord which may be rehydrated for use following a lyophilization process.

Yet another object of the present invention is to provide a preserved vessel derived from a human umbilical cord which, when

implanted, provides a supply of blood and nutrients to human tissue.

Another object of the present invention is to provide a preserve vessel derived from a human umbilical cord which may be implanted safely, obviating harvesting of veins from a patient during certain surgical procedures.

Another object of the present invention is to provide a preserved vessel derived from a human umbilical cord possessing uniform width, and lacking tributaries and valves.

A further object of the present invention is to provide a plentiful supply of preserved vessels derived from a human umbilical cord for implantation.

The invention possesses other objects and advantages especially as concerns particular characteristics and features thereof which will become apparent as the specification continues.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a side elevation view of a raw vascular segment from a human umbilical cord.

Fig. 2 is a side elevational view depicting the raw umbilical cord vascular segment having a stent placed within its lumen.

Fig. 3 is a top plan view of the combined vascular segment and stent within a gauze carrier.

Fig. 4 is a side elevational view of a freeze-drying canister containing the lyophilized vascular segment, stent, and gauze carrier depicted in Fig. 3.

For a better understanding of the invention references made to the following detailed description of the preferred embodiments which should be referenced to the prior described drawings.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Various aspects of the present invention will evolve from the following detailed description of the preferred embodiments thereof which should be referenced to the prior described drawings.

The preserved vessel of the present invention 10 is obtained from human placenta having an attached umbilical cord following child birth. It has been found that umbilical cords serve as an advantageous source of vessels for ultimate implantation since umbilical cord vessels lack branching, are generally of uniform cylindrical width, and obtain no one-way valves, as do saphenous veins. The human umbilical cord contains two arteries and one vein, each of which are suitable for implantation. The placenta and umbilical cord unit is refrigerated, but not frozen or placed in a preservative, for recovery of the vessels within a six hour period. Cooling is maintained during transportation to the harvesting site.

The donor unit is received at a processing center, where three separate and sequential processing stations are prepared, each using sterile environment, instruments, and technique. At the first station the umbilical cord and placenta are grossly rinsed, blot dried briefly with absorbent towels, and then placed at the second station with the umbilical cord elongated.

At the second station the umbilical cord and placenta are inspected to insure that the cord contains two arteries and one vein. Any two-vessel umbilical cords are not utilized in the present invention. The point of dividing the umbilical cord from

the placenta will varying according to length and size needs. In general, however the umbilical cord will be transversely incised at a point 0.6-cm from entry into the placenta. The processing of the umbilical cord at the second station involves identifying the ends of the arteries and veins and irrigating each vessel using smooth cannulation, with a heparin solution. This process physically removes fetal blood within the vessels and chemically inhibits clot formation from remaining platelets. The umbilical cord is then incised at one end. Using blunt and sharp dissection techniques, the vessels are separated from the sheath along the interposed Wharton's jelly. The arteries are easily identified from its helical arrangement around the thicker wall umbilical vein. The three vessels are then bluntly detached from one another such that the arteries maintain a serpentine configuration. The vessels are then incised transversely to provide varying vessel lengths as required.

Fig. 1 represents a vascular segment 12 at this point in the recovery process. Vessel 12 is then fitted with a nylon stent 14 which passes through the lumen or passageway 16 of vessel segment 12, Fig. 2. Vessel 12 and stent 14 are then placed in a moist gauze carrier 18, Fig. 3. At this point, the vessels within the umbilical cord, such as vessel 12, have been protected from freezing, contamination, rough handling, and desiccation. The unit 20, Fig. 3, consisting of the vessel segment 12, stent 14, and gauze carrier 18, is then positioned or placed within a canister 22, depicted schematically in Fig. 4. Canister 22 is sealable and

capable of holding a vacuum. Needless to say, canister 22 is sterilized. Canister 22 may be refrigerated temporarily pending transfer to a commercial freeze drier. Each canister, such as canister 22, is marked to identify tissue origin and to record the length, size, and branching, if any. Canister 22 is then placed in a freeze drier, such as a Virtis freeze drier, and freeze-dried. Typically this process takes place between minus 35 degrees centigrade and minus 60 degrees centigrade under a vacuum of, typically, 0.4 atmospheres, and over a 24 hour period. Such freeze-drying process allows removal of 90% of the water in vessel segment 12 through the process of sublimation of the same. Thus, damage to the structural integrity of vessel segment 12 is avoided and, it is theorized that, freeze-drying process destroys the antigen responsible for ultimate rejection of the graft in a human. When the canister 22 and unit 20 containing the lyophilized vessel segment 10 may be stored for a relatively long period of time, measured in years. During such storage, the canister 20 is left intact, including the maintaining of a vacuum within chamber 24 of canister 22.

A third station would receive the placenta with its umbilical stump. At this station, the remaining portions of the placenta and umbilical cord sheath stump may be employed for other further use.

At the time of graft utilization, preserved vessel 10 and canister 22 would be selected, based on the recipients needs with a patient under anesthesia. The patient's recipient site is

exposed surgically. Under continuing sterile technique, canister 22 is opened by surgical attendants and the sterile unit 20 containing the freeze-dried preserve vessel 10 is removed. The vessel 10 and gauze carrier 18 are irrigated with saline solution for five minutes. The vessel is then removed from the stent 14 and the gauze carrier 18 for use and irrigated with heparin solution. Vessel 10 is sutured at each end, as it is implanted in the patient's recipient site, utilizing standard vascular anastomosis technique. Following release of vessel clamps of the recipients vessel, the surgeon would observe the flow and patency of the vessel to assure integrity of the graft.

While in the foregoing, embodiments of the present invention have been set forth in considerable detail for the purposes of making a complete disclosure of the invention, it may be apparent to those of skill in the art that numerous changes may be made in such detail without departing from the spirit and principles of the invention.

The following Examples are illustrative of the invention sought for patenting, but should not be deemed to limit the invention in any manner.

EXAMPLE I

Grafts consisting of polytetrafluoroethylene (PTFE) in the form of tubes having a 1.0 mm internal diameter where carried out in 18 Sprague-Dawley rats. Each graft was 1 centimeter in length. The grafts were placed in one femoral artery of each rat

to supply a 3x3 centimeter skin flap of the rat abdomen. The skin flap served as a visual indicator of graft patency.

Each rat was anesthetized with intraperitoneal pentobarbital having a concentration of 50 mg/kg. Each rat was secured on an operating board to allow access to the abdomen and inguinal areas. The abdominal area of each rat was shaved and antiseptic technique was used. A 3x3 cm abdominal skin flap (epigastric island flap) was incised and hemostasis was obtained. The femoral artery and vein, and their branches, as well as, the epigastric artery and vein for each rat, where identified. The epigastric vessels supplied blood to the skin flap. The venous system was left in tact. The femoral artery, proximal to the epigastric branch, was clamped with two vascular clamps, with excision of a 5-mm arterial segment. The arterial ends were irrigated with heparin solution (300 U/ml). Although the internal diameters of both the PTFE graft and the femoral artery where one centimeter, the significantly thicker walls of the PTFE graft required placing a continuous suture in a manner that would leave the end of the femoral artery within the lumen of the PTFE graft. A telescoping suture technique was used. The distal portion of the PTFE graft was then anastomosed to the distal portion of the femoral artery in a similar manner. The clamps were then released and the blood flow through the graft and into the epigastric pedicle was observed. The graft and abdominal skin where observed for five minutes. The "flicker test" was used to demonstrate flow in the superficial epigastric artery by gently elevating forceps on

the undersurface of the artery and demonstrating pulsatile blood flow to that artery. Such blood was supplied through the emplaced PTFE graft. The skin was then sutured.

Two rats served as controls, in which the epigastric island flap was isolated and the epigastric artery was ligated, without placement of the PTFE graft. The flaps of the control animal demonstrated necrosis within 24 hours. The 18 rats with PTFE grafts demonstrated 50% patency. Sections of the grafts were taken for histologic and electron microscopy studies. Electron microscopy endothelial growth into the lumen of the PTFE graft from each end was noted. In essence, the telescoping suture technique accomplished the goal of anastomosing the dissimilar vessel ends, but the anastomosis technique was difficult to accomplish. Undesirably, the PTFE grafts remained rigid and served as rigid conduits. A PTFE graft of a longer length was seen to kink, which would preclude utilization of longer lengths of the graft, i.e., around a joint. However, this study indicated that endothelial proliferation through the lumina of the graft from each end occurred. It was concluded that a better graft material was needed.

EXAMPLE II

17 Sprague-Dawley rats were utilized to develop epigastric island flaps, based on the inferior epigastric vessels which branch from the femoral vessels detailed hereafter. 18 femoral vein segments from other rats were harvested for freeze-drying. To accomplish this task, each rat was anesthetized with

interperitoneal Nembutal (50 mg/kg). Each rat was placed supine on a rat board and the abdominal skin was shaved. Using antiseptic technique and with the abdomen prepped, the right and left femoral arteries were identified and clamped. A ten millimeter length of vein from each side was removed. The rats were then euthanized according to accepted humane techniques. The removed femoral grafts were irrigated with heparinized saline (300 U/ml). A nylon stent was then placed through the lumen, enclosed in a moist gauze carrier, and placed in a 10-cc freeze-dry vial. In this container, the grafts were then freeze-dried on a Virtis bench top, three model freeze-drier, manufactured by the Virtis Company, Inc. of Gardiner, New York. Freeze-drying was carried out over 24 hours at 0.4 atmospheres of pressure and at a temperature of minus 70°F through the process of sublimation. In this process, the water within the specimen passes from a frozen crystalline state into the vapor state without expansion which occurs in the liquid state. Thus, the disruption of the integrity of the cellular structure of the vessel was avoided. Each specimen was stored at room temperature in the freeze-dry vial or canister with the vacuum seal being maintained.

The 17 Sprague-Dawley albino male rats weighing between 350 and 500 grams were then utilized for the femoral vein study. Each rat was anesthetized with intraperitoneal Nembutal (30 mg/kg) placed on a rat board, and the abdomen and groin were shaved. Using antiseptic technique, a 3x3-cm abdominal skin flap based on the epigastric artery and vein (epigastric island flap) was

dissected. The femoral artery and vein and the epigastric vessels to each island flap were identified. The venus system was left intact. Two vascular clamps were placed on one femoral artery. A 6-mm arterial segment was excised. The cut ends of the femoral artery were then irrigated with heparinized saline (300 U/ml). The freeze-dried vessel was then obtained from one of the freeze-dried canisters, rehydrated with saline for 30 minutes, and anastomosed at the proximal cut end of the femoral artery using eight 10-0 Ethilon sutures under magnification, with a Carl Zeiss Opmi-6-SD microscope. The distal anastomosis was completed in a similar manner. The clamp was then removed and blood was seen to flow through the vein graft into the epigastric artery. Patency was demonstrated using the "flicker test" as well as by the milking (strip) test. The femoral artery distal to the epigastric artery was then ligated. This technique allowed blood to flow through the freeze-dried graft, which had been placed as the sole arterial blood supply to the abdominal flap. The abdominal skin flap was then closed with a running 4-0 Vicryl suture. The flaps were then observed to be pink.

After two months, allowing enough time for collateral circulation to develop into the flaps, the patency of the vessel was demonstrated by anesthetizing the rat, with careful dissection through a groin incision to demonstrate the freeze-dried vessel segment. By observation, flicker and strip tests patency was determined. The patency rate was calculated to be 66%. Histologic and electron microscopy studies were carried out, demonstrating the

presence of minimal intimal hyperplasia where the endothelial cells enter the graft lumen at each end from the femoral artery. Endothelialization was demonstrated as a thin epithelial layer within the lumen. Also, neovascularization of media and adventitia, as well as proliferation of fibroblasts, adjunct to the rebuilding of the vessel wall, were demonstrated. There was no evidence of graft rejection. The vein graft was noted to propagate the pulse visually and flicker and strip tests were readily demonstrated. Thus, the freeze-dried vessels were seen to undergo remodeling in vivo by a normal reparative process without evidence of cellular immune response.

EXAMPLE III

15 Sprague-Dawley albino male rats were utilized to demonstrate the use of freeze-dried femoral arterial allografts. The arterial grafts were obtained from eight additional rats, each of which were anesthetized with intraperitoneal Nembutal (50 mg/kg) and then placed on a rat board. The abdominal and groin skin were then shaved and prepped. Antiseptic technique was utilized. The femoral arteries were identified, isolated by blunt and sharp dissection and then clamped to permit excision of a 1.0-cm section of each femoral artery. The grafts were then irrigated with heparinized solution (300 U/ml). A nylon stent was then placed in the lumen of each artery and the graft and stent were placed in a moist gauze carrier. A 10-mm vial or canister was then used to contain the graft, stent and carrier unit. The graft unit was then freeze-dried over 24 hours according to the parameters described in

Example II. The graft specimens were then stored on a shelf at room temperature maintaining the vacuum in the canister. The animals used for the donor vessels were subsequently euthanized in accordance with humane standards.

The 15 study animals heretofore described, were then utilized and prepared. The Sprague-Dawley rats, each weighing between 430-570 grams, were given intraperitoneal Nembutal (50 mg/kg) and placed on a rat board. The abdominal and groin areas were then shaved and antiseptic technique was utilized. With respect to each rat, a 3x3-cm epigastric island skin flap was incised and elevated, based on the epigastric artery and vein branching from the femoral vessels. Cardiovascular clamps were then placed on the femoral artery, permitting excision of a 6-mm arterial segment. The cut ends of the femoral artery were then irrigated with heparinized saline (300 U/ml). A freeze-dried artery was then obtained from the shelf canister or vial for each rat specimen. The artery was rehydrated in saline for 30 minutes. Proximal anastomosis was then completed with eight 10-0 Ethilon sutures under magnification. The individual sutures were placed sequentially in a circumferential manner for the anastomosis. The distal anastomosis was similarly completed. The clamps were then removed and circulation through the graft was noted, including circulation into the epigastric artery using flicker and strip testing. The femoral artery distal to the inferior epigastric artery was then ligated. The sole arterial supply to the graft was noted to be through the freeze-dried graft. After rechecking

patency the abdominal wound was closed with 4-0 Vycryl sutures. The observation of the abdominal flap demonstrated the presence of viability, indicative of graft patency. Although there was one kennel death from unrelated causes during a two month period, grafts in the 14 remaining rats exhibited a visual patency rate of 93% (13 of 14 flaps remaining visibly viable). The rats were anesthetized with interperitoneal Niembutal (50 mg/kg), placed on a rat board, and the groin was shaved. An incision was carried down to the level of the graft, with 13 grafts being viable. After demonstration with the flicker and strip tests, sections of the graft were then recovered for histologic and electron microscopy studies the grafts were noted to have developed endothelial lining, stemming from each end of the graft. The vessel walls demonstrated normal healing processes. There was no evidence of graft rejection. The arteries were found to be easier to handle, being thicker walled facilitating the anastomosis. There was no evidence of aneurysmal dilatation.

EXAMPLE IV

20 New Zealand white female rabbits weighing between 2.6 and 5.8 Kg were utilized to study freeze-dried arterial grafts. Grafts were initially obtained from five other rabbits in which two femoral and two brachial arteries were harvested from each rabbit. The harvesting began with intermuscular Ketamine (50 mg/kg) and Xylazine hydrochloride (20 mg/kg) as an animal tranquilizer. Each animal was then placed under general anesthesia and maintained by oxygen and halothane delivered by mask. Each rabbit was placed on

a board and the groin was shaved. Using sterile technique. Femoral and brachial arteries were identified and harvested in 3.5 to 4.0-cm vessel lengths. The grafts were then irrigated with heparinized saline (300 U/ml). A nylon stent was placed in each graft lumen. The graft and stent were placed in a moist gauze carrier, and then positioned in a 15 ml vial. The specimens within the vile were then freeze-dried over 24 hours, according to the techniques described in Example II.

Following freeze-drying, the samples were stored on a shelf at room temperature maintaining the vacuum seal in the canister. The studied rabbits were then anesthetized in the before mentioned manner, and placed on a board. One femoral artery was identified through a longitudinal groin incision. A 2.0-cm segment of the femoral artery was excised, leaving a 3.5-cm defect secondary to vessel retraction from elastic recoil. The cut ends of the arteries were then flushed with heparinized saline (300 U/ml). Six of the rabbits received a brachial artery each having an internal diameter of 2 mm. Ten rabbits received femoral artery grafts, each having a 2.3 mm internal diameter. The grafts were then anastomosed as interpositional grafts using 10.0 Ethilon sutures under magnification. Clamps were then removed and the patency of the grafts was demonstrated visually through flicker and strip tests. Four other rabbits were used as a control group in which a 3.5-cm segment of femoral artery was removed and replaced as an autograft. Due to retraction, the defect following removal of the 3.5-cm segment was 4.5-cm. With reanastomosis of the

sections, greater tension on the suture line was noted. The microvascular clamp were removed and patency was noted. In each rabbit, the skin incision was closed with running 4.0 nylon sutures. The six rabbits receiving brachial grafts exhibited no patency. Of the ten rabbits receiving femoral grafts, five exhibited patency. The four control animals exhibited no patency. The results of histologic and electron microscopy studies indicated the patent femoral arteries possessed neo-endothelization stemming from each end of the graft. There was no evidence of lymphocytic infiltration in the wall of any graft. Fibroblastic and myoblastic infiltration of the cell wall was noted as a normal reparative process. There was no evidence of immune response. It is concluded that the rabbit model proved more difficult than the study of the rat models of Examples II and III.

EXAMPLE V

A human placenta and an attached umbilical cord was received five hours post delivery. This tissue was placed in a plastic container or a bucket with an overlying moistened surgical towel. The human placenta was removed from the plastic container, rinsed with saline, and positioned on a dissection board. The cut ends of the two arteries and veins within the umbilical cord were easily identified and cannulated. Each vessel was then irrigated with heparinized saline (300 U/ml), to clear the vessels of blood and clots and to prevent additional clotting by blood platelet remnants. The umbilical cord was laid out on an operating board. 6-power loupe magnification was employed in this regard. A

longitudinal incision was made over the entire length of the umbilical cord through the outer sheath. The sheath was isolated from the vessels by dissection, bluntly, through the Wharton's Jelly and, sharply, by the incision of the attaching fibrous bands which extended from the vessel bundled to the sheath. The edges of the sheath were superiorly pinned to the board using marker pens with one rounded plastic end. The tissue was irrigated with saline every two minutes. In a similar manner, the lower edge of the sheath was separated from the vessels by blunt and sharp dissection techniques and pinned to the operating board. The three vessels were easily identified to spiral in a counterclockwise manner. The superficial portions of each vessel were then separated by blunt and sharp dissection at the peak of each spiral. A gentle spreading technique was employed to penetrate tissue planes between the vessels while the transverse fibrous bands were cut intermittently. Each vessel was freed circumferentially and a loop was passed beneath the same to facilitate gentle traction of the vessel. Each vessel loop consisted of a colored plastic strand, 1 mm in diameter. Progressive freeing of the vessels was accomplished with continuing intermittent irrigation to maintain an acceptable moisture level. The vessels were then separated from each other and from the sheath. The fibrous band and sheath of the umbilical cord were also isolated. All in all, two veins, one artery, one fibrous band, and the sheath were presented for freeze-drying. The vessels were handled with a nylon stent placed within the lumen to maintain vessel diameter following re-irrigation with

heparinized saline. The stents minimized direct handling of the vessel and acted as a carrier for the vessel during the freeze-drying and rehydration phases. The stents also accommodated vessels of differing resting diameters and allowed the capability of tapering of one end of the vessel for anastomosis of dissimilar ends. In addition, the vessels may be incised transversely to provide specific required lengths.

The vessels, sheath, and band are placed in individual canisters and key coded for identification. Vessels and enclosed stents are covered by a moist gauze carrier and placed in the freeze-dried canister for lyophilization. Lyophilization takes place according to the steps of Example II. The vessels are stored, following lyophilization, on a shelf at room temperature for subsequent use. Rehydration of the vessels requires removal from the canisters, soaking in heparinized saline for 20 minutes and removal of the stents from the cotton gauze carrier. Implantation of the vessel takes place following removal of the stent and trimming of the vessel to the size needed for implantation using standard vascular anastomosis techniques.

WHAT IS CLAIMED IS

1. A preserved vessel isolated from a human umbilical cord and lyophilized for use as an allograft to improve blood supply to human tissue, said preserved vessel exhibiting low antigenicity.

2. The preserved vessel of claim 1 comprising a vein.

3. The preserved vessel of claim 1 comprising an artery.

4. The preserved vessel of claim 1 which additionally comprises a stent located in the lumen of said preserved vessel.

5. The preserved vessel of claim 4 in which said preserved vessel and stent further include a canister under vacuum for containing said preserved vessel and stent.

6. The preserved vessel of claim 1 in which said isolated vessel is substantially free of fetal blood through irrigation.

7. The preserved vessel of claim 1 which comprises a straight vessel segment.

8. The preserved vessel of claim 1 which comprises a branching vessel segment.

EXPERIMENTAL FREEZE-DRIED MICROARTERIAL ALLOGRAFTS IN RABBITS

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ABSTRACT

Arterial allografts of 3.5 cm length were freeze-dried and placed into the femoral arteries of 20 female rabbits (16 experimental subjects and four controls). Immediate patency was 100%. Subjects were surgically explored after two months of observation. Overall patency at this time was 31% (5/16). A patency rate of 50% (5/10) was achieved with size-matched femoral grafts. However, all of the smaller diameter brachial grafts were unsuccessful. Analysis by light microscopy as well as transmission and scanning electron microscopy demonstrated intimal hyperplasia which was more prominent in nonpatent grafts. There was no evidence of a cellular immune response to the freeze-dried grafts by the host. The use of size-matched grafts and postoperative anticoagulants in future studies may improve patency rates and the potential clinical applicability of this promising microvascular technique.

INTRODUCTION

Microvascular techniques offer important alternatives for head and neck reconstruction following major tumor resections. Free flap transfers, although not yet commonplace, provide excellent tissue coverage for large defects. When this method of reconstruction is selected, the head and neck surgeon may be faced with inadequate vascular pedicle lengths or the need to anastomose vessels outside irradiated tissue beds. This procedure may require the use of interpositional vascular grafts.

In 1977, Biemer[1] used autogenous veins, which thereafter represent the gold standard against which all synthetic or preserved microvascular substitutes must be measured. There are, however, some notable limitations to their usage. For example, in certain patients, potential donor sources for venous autografts may be reduced by prior intravenous chemotherapy or the presence of obliterative vascular disease. In addition, intraoperative vessel procurement can be long, tedious and unproductive, especially in debilitated, elderly patients. Finally, there is increased morbidity associated with additional incisions and a longer procedure. It is beneficial, therefore, to have reliable prosthetic or preserved vascular grafts available for clinical use.

Laboratory studies of Broncon et al,[2] Ganske et al,[3] and Coffee,[4] investigating synthetic microvascular substitutes (primarily polytetraflouroethylene), have been inconclusive and inconsistent. On the other hand, recent reports by Chow et al[5] and Pratt et al[6] on freeze-dried vascular micrografts have been quite encouraging. Patency rates as high as 93% have been described with freeze-dried microarterial grafts in the rat model by Pratt et al.[7]

Autogenous venous micrografts have been used reliably in the rabbit by

Fujikawa and O'Brien,[8] and Dac et al.[9] Melka et al[10] achieved better success with microarterial autografts when compared to microvenous autografts in both the rabbit and the rat. Their study described superior long-term results with the microarterial grafts. In addition, Chow et al[11] had some success using freeze-dried human placental vessels as heterografts in the rabbit. All of these investigations have produced data on short-vessel segments, most commonly measuring one cm or less. If a potential microvascular substitute is to be clinically acceptable, it must be demonstrated to be reliable in longer graft segments. The present study was designed to investigate the immediate and the long-term patency in addition to the host tissue response using 3.5 cm freeze-dried microarterial interposition allografts in the rabbit model.

MATERIALS AND METHODS

Femoral and brachial arteries were harvested in 3.5 to 4.0 cm lengths from New Zealand white female rabbits. All branches from each segment were suture ligated and incised. These donor arteries were lyophilized slowly over a 24 hour period in the Virtis Bench Top 3 Model Freeze Dryer (The Virtis Co., Inc., Gardiner, NY) and stored in 15 ml vacuum sealed vials at room temperature. Prepared vessels were rehydrated in normal saline (50 ml) for 20 minutes before grafting.

The subjects in the study were New Zealand white female rabbits weighing between 2.6 and 5.8 kg. General anesthesia was induced with IM Ketamine (50 mg/kg) and Xylazine HCl (20 mg/kg), an animal tranquilizer. Anesthesia was maintained with oxygen, and halothane was delivered by mask. The groin was shaved, and a surgical depilatory was applied to the area. Using aseptic technique, the femoral vessels were exposed on the right side through a

longitudinal incision in the medial thigh. The femoral artery (2.2 to 2.3 mm internal diameter) was topically irrigated with 1% lidocaine to relieve spasm.

There were two experimental groups, one received the brachial grafts and the other received the femoral grafts. In both experimental groups, microvascular clamps were placed and a 2.0 cm segment of the femoral artery was excised. This left a 3.5 cm defect secondary to vessel retraction from elastic recoil. The cut ends of the arteries were then flushed with heparinized saline (330 units/ml), and the adventitia was trimmed. The previously prepared freeze-dried brachial (1.8 to 2.0 mm internal diameter) and femoral (2.2 to 2.3 mm internal diameter) arteries were used as interposition grafts (Fig. 1). End-to-end anastomoses were performed with 10-0 Ethilon suture on a BV75 needle using the Carl Zeiss OPMI-6SD microscope. Eight interrupted sutures were used to perform each anastomosis with the freeze-dried graft. Average ischemia time was 50 minutes in experimental subjects (range 35 to 90 minutes).

In the control group, a 3.5 cm segment was removed and used as an autograft. As a consequence of elastic retraction of the vessel walls, a 4.5 cm defect was created. Therefore, each control subject had a 3.5 cm graft placed into a 4.5 cm defect, whereas each experimental subject had a 3.5 cm graft placed into a 3.5 cm defect. Twelve sutures were required in the control anastomoses in order to overcome the high tension generated by vessel retraction. Average ischemia time was 70 minutes in control subjects (range 50 to 90 minutes).

Microvascular clamps were removed and immediate patency at each anastomosis was assessed by standard techniques. These included the flicker test, strip test and the presence of expansile pulsation. The skin incision was closed with running 4-0 Vicryl sutures.

The groin was surgically explored at two months, and the femoral vessels were examined. Long-term patency was determined at this time by incising the femoral artery distal to both anastomoses and observing for brisk blood flow. Perfusion fixation of the grafts was performed with glutaraldehyde solution using the technique of Derman and Schenk.[12]

Grafts were harvested, analyzed and each specimen was sectioned longitudinally. One half was prepared for transmission electron microscopy evaluation and specimens were osmicated, dehydrated, embedded in Medcast and stained with uranylacetate and lead citrate. Segments were stained with toluidine blue and examined under a light microscope. The other half was evaluated by scanning electron microscope and specimens were osmicated, dehydrated, critical point dried and gold coated.

There were 21 subjects in the sample group. These subjects comprised one control group and two experimental groups. Of the experimental groups, one contained six brachial graft subjects and the other contained 11 femoral graft subjects. There were four subjects in the control group. One experimental subject from the femoral group died of anesthetic complications following successful graft interposition and was excluded from the study. This left a total of 16 experimental subjects and four control subjects for evaluation.

RESULTS

All grafts were patent immediately following the surgical procedure. At two months, the overall patency rate was 31% (5/16) for the experimental group and 0% (0/4) for the control group. Although none of the smaller diameter brachial grafts (0/6) were patent at the time of exploration, 50% (5/10) of the size-matched femoral grafts were patent and exhibited brisk flow when the distal femoral artery was incised. The nonpatent grafts were surrounded by

extensive fibrosis. Seven of the nonpatent grafts were flat and attenuated suggesting early failure; however, three grafts were round and contained firm, well organized thrombus formations. There was one occlusive aneurysm in a nonpatent graft. The five patent grafts were surrounded with minimal fibrosis.

Descriptions of microscopy data will be based on the presence or absence of patency. Light microscope examination of the patent grafts revealed minimal intimal hyperplasia in the native femoral artery which was continuous with a neointimal lining in the freeze-dried graft. There was no evidence of lymphocytic infiltration in the wall of any graft. Increased intimal hyperplasia was noted in nonpatent grafts.

Examination of patent grafts by electron microscopy revealed a single layer neointima with a mature tunica media (Fig. 2). The endothelium of the grafts took on the appearance of the native vessel (Fig. 3), except that it had an undulating surface (Fig. 4). The elastic membrane was highly organized. The tunica media contained an abundance of active cells (fibroblasts and myoblasts) with prominent rough endoplasmic reticulum. No lymphocytes were discovered in the wall of any graft. Endothelial pseudopodia from the native femoral artery projected over the graft surface to form a neointimal lining (Fig. 5). These findings correlated well with data obtained by light microscopy.

The data were analyzed by applying two statistical tests. Due to the error introduced by creating a larger arterial defect in the control rabbits, this group was deleted from statistical analysis. Patency rates from the two experimental groups were compared using the Fisher Exact Probability Test. Differences between the results obtained in the brachial and femoral groups were not statistically significant. However, a definite trend was noted

($p = .06$).

The data from the femoral group were then analyzed using the binomial test which compares experimental results to an empirical norm. As previously stated, the autogenous vein graft is the standard against which all vascular substitutes must be compared. Fujikawa and O'Brien achieved 84% patency (16/19) with 40 mm interpositional femoral vein autografts placed into rabbit femoral arterial defects. As their's is the only published study of longer microvenous autografts in rabbits, their patency rate was used as an empirical norm. There was insufficient published data to establish a theoretical norm. Using this value (84% patency), the null hypothesis, which stated there was no statistical difference between obtained results and the established empirical norm, was rejected ($p < .01$, one tailed). Stated otherwise, a statistically significant difference was established between the freeze-dried femoral artery allografts used in this study and microvenous femoral allografts which produced higher patency rates in Fujikawa's study using the same animal model.

COMMENT

The results of this study revealed several potential problems with the use of freeze-dried microarterial grafts. The patency rates were unsatisfactory, and this observation will be analyzed in depth. The intimal hyperplasia, more prevalent in the nonpatent grafts by microscopy, is well described by Berguer et al.[13] This is the expected tissue response to acute vascular injury and it is enhanced by low blood flow states induced by thrombogenesis. There was no evidence of an observable cellular immune response according to the microscopy studies; micrographs indicated no lymphocytic infiltration in graft walls. This finding could represent a very beneficial characteristic of freeze-dried vascular grafts but it bears further investigation. Each of these points will now be discussed in detail.

Preserved microvascular substitutes would be beneficial to the head and neck surgeon as long as their patency rates approach those of microvenous interposition autografts (80 to 90%), as reported by Biemer[1] and Fujikawa and O'Brien.[8] Surgical technique, low blood flow states and thrombogenicity of the graft material affects the potential for thrombosis. In small diameter vessels (2 mm and below), Barnes[14] states that a minute change in internal diameter can substantially reduce blood flow rates and lead to occlusion. Normal endothelium is resistent to thrombosis; however, and Ackland[15] notes that endothelium interrupted at a suture anastomosis enhances thrombosis and potential graft failure. This occurs when platelets are activated and chemotactic factors are released thereby initiating thrombogenesis. In order to prevent vessel thrombosis, it has become a common practice for clinical microvascular surgeons to use postoperative antiplatelet and anticoagulant therapy. Kolar et al[16] have reported on improved patency in coagulated rabbits. In 1974, Kogma et al[17] revealed that the mechanism for the blood coagulation in the rabbit is quite similar to that in the human, and it seems apparent that the success of investigations with freeze-dried micrografts in rabbits may be improved by incorporating antiplatelet and anticoagulant therapies. This was not done in our study.

Surgical technique, another important variable to consider when evaluating patency rates, was relatively constant. Each procedure was performed by two experienced microvascular surgeons working as a team. The only procedural differences were related to the graft material. There was a notable size mismatch between the native femoral arteries and the freeze-dried brachial grafts which were not readily distensible. The placement of these smaller, nondistensible grafts would be expected to reduce blood flow, increase

turbulence at each anastomosis and thereby increase thrombogenic potential. Although statistical analysis suggests no difference between the brachial and femoral grafts, the clinical significance of the difference in patency is apparent. Since technique was relatively constant, the only variable between these groups was the internal diameter of the vascular grafts. It is logical, therefore, to consider this an important factor in explaining the vastly different patency rates.

The control procedures, which were all unsuccessful, contained one important difference in comparison with experimental procedures. Since the control autografts were placed into defects enlarged by the normal retraction of vessel ends, tension across each anastomosis was considerably increased. In the experimental procedures, a 2.0 cm segment of femoral artery was excised, leaving a 3.5 cm defect influenced by intrinsic elastic retraction, into which a 3.5 cm freeze-dried graft of identical length was interposed. Thus tension was minimized in the experimental subjects. This was not so in the control subjects, which had a 3.5 cm graft placed into a 4.5 cm defect. The increased tension necessitated the use of more sutures to complete each control anastomosis. These factors selectively increased thrombogenicity and created bias in the control group. This represented a weakness in the study. A better control design would have utilized grafts taken from the contralateral femoral arteries. These autografts could then have been placed into defects which were identical to those created in the experimental subjects. Even though this would have involved an additional groin incision in control subjects, procedural bias would have been minimzed.

Bergner et al[13] have reported that intimal hyperplasia is a common cause of failure in microvascular grafts. Low blood flow rates are associated

with more extensive intimal hyperplasia which can cause occlusion. Many of the nonpatent grafts in this study showed prominent intimal hyperplasia as expected. The patent grafts had no evidence of this histopathology but rather had smooth, single-layer intimal linings. These data support similar findings recently reported by Pratt et al[7] with freeze-dried microarterial grafts.

Review of the micrographs showed no observable evidence of lymphocytic infiltration in the freeze-dried graft walls. As was reported by Pratt et al,[7] this finding suggests the absence of a similar immune response to the freeze-dried microarterial grafts by the host. A stronger statement about the lack of immunogenicity could be made by analyzing and quantifying the host immune response to these grafts with immunohistochemical studies in future investigations.

There was a significant difference between results obtained with freeze-dried microarterial grafts and the results expected using an empirical norm (microvenous autografts). In evaluating these data, it is important to consider all of the limitations mentioned in this discussion. The use of size matched arterial interposition grafts, as well as postoperative antiplatelet and anticoagulant therapies in future studies may improve patency rates. The introduction of an improved control design would also assist with data interpretation. Immunohistochemical studies to further document the absence of a cellular immune response to the freeze-dried grafts would be beneficial.

With the limitations of the present study notwithstanding, this new microvascular technique shows promise. Eventually, it is anticipated that freeze-dried placental or donor vessels could be used in clinical trials if future preliminary laboratory studies in rabbits and larger animals are successful.

ACKNOWLEDGEMENTS

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LEGENDS

Figure 1. Freeze-dried microarterial interposition graft placed into the right femoral artery through a longitudinal groin incision.

Figure 2. Transmission electron micrograph of a freeze-dried artery harvested at two months showing a patent lumen (L), a single layer endothelium, viable fibroblasts (F) and mature collagen (C). (original magnification x 1,900)

Figure 3. Scanning electron micrograph (SEM) showing smooth proximal endothelium similar to native artery. Patent lumen contains RBC clot (arrow) not attached to vessel wall - artifact of fixation. (original magnification x 20)

Figure 4. SEM appearance of undulating endothelial surface (arrow) in a freeze-dried arterial graft. (original magnification x 200)

Figure 5. SEM showing endothelial pseudopodia from the proximal native artery projecting onto the inner surface of the freeze-dried graft. (original magnification x 5,000)

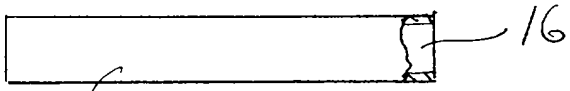

FIG. 1

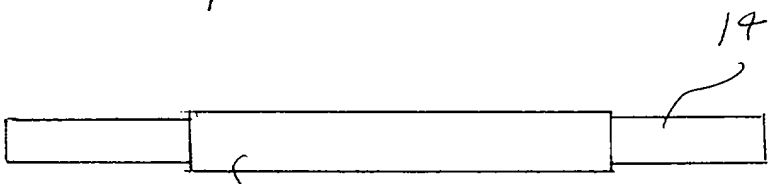

FIG. 2

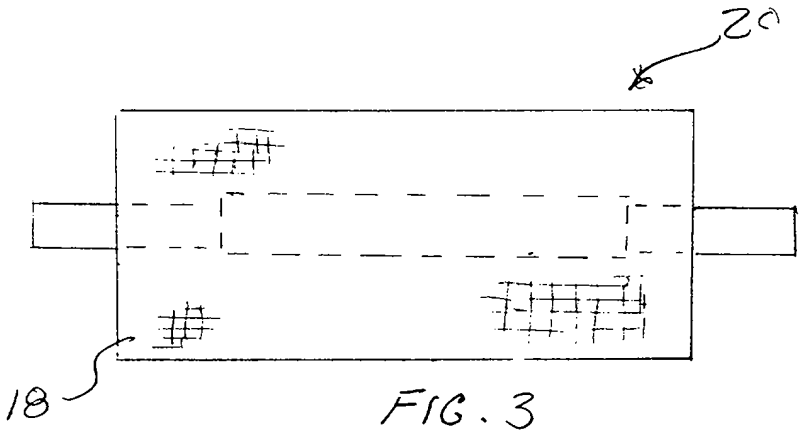

FIG. 3

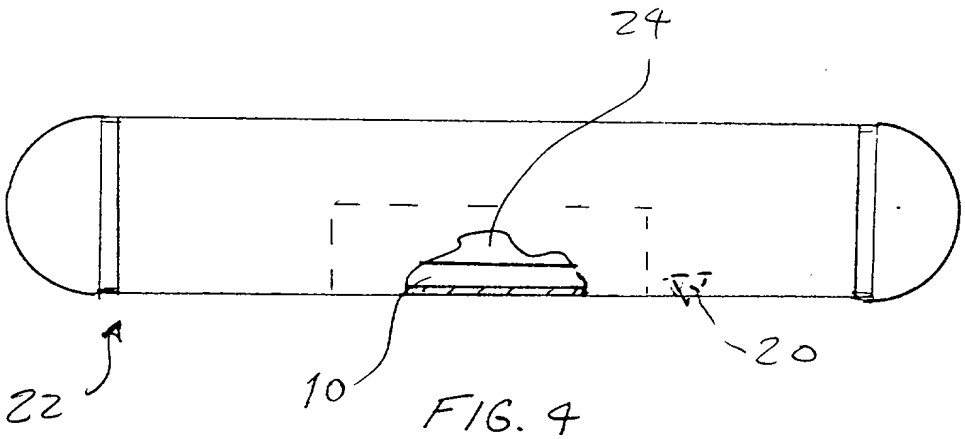

FIG. 4

COMBINED DECLARATION AND POWER OF ATTORNEY

IN ORIGINAL APPLICATION

Atty Dkt. No: **13172**

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

A PRESERVED IMPLANTABLE VESSEL DERIVED FROM A HUMAN UMBILICAL CORD

the specification of which **X** is enclosed herewith or __ was filed on _____ as Application Serial No. _____ and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign applications(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Applications(s):

COUNTRY _____ APPLICATION NUMBER _____

DATE OF FILING _____ PRIORITY CLAIMED UNDER

35 U.S.C.119 YES _____ NO _____

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35 U.S.C.119 YES _____ NO _____

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37 Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

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APPLICATION SERIAL NO. _____ DATE OF FILING _____

STATUS: ____ PATENTED ____ PENDING ____ ABANDONED

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I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Serial or Patent No: Docket No: **13172**
Filed or Issued:
For: **A PRESERVED IMPLANTABLE VESSEL DERIVED FROM A HUMAN UMBILICAL CORD**

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[**X**] the specification filed herewith
[ ] application serial no._____, filed_____
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF INVENTOR: **James R. Schneider**

Signature of Inventor: James R Schneider, M.D.

Date: Aug. 7, 1998